

INTERCELLULAR UPTAKE OF TECHNETIUM-99M PERTECHNETATE BY DIFFERENT TYPES OF CELL LINES

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ABSTRACT

The purpose of this study is to determine the technetium-99m pertechnetate ($^{99m}\text{TcO}_4$) intercellular uptake by different types of cell lines. HeLa, human fetal osteoblast (hFOB), glial and glioma cell lines grown in 6-wells culture plates were incubated with $^{99m}\text{TcO}_4$ of activity of 200, 400, 600, 800 and 1000 μCi for 30 minutes at 37°C and 5% CO_2 humidified atmosphere. After incubation, the cells were washed 3 times with phosphate buffer saline to remove the extracellular traces of $^{99m}\text{TcO}_4$. Measurements of the intercellular $^{99m}\text{TcO}_4$ radioactivity were performed using single head gamma camera and the percentage uptake of the $^{99m}\text{TcO}_4$ into the cells was calculated. The intercellular uptake of $^{99m}\text{TcO}_4$ was found to be inversely correlate to the radioactivity. HeLa cell shows the highest uptake followed by hFOB, glial and glioma cell lines. Comparison of uptake between normal and cancer cells present indistinguishable results. The findings of this study suggest that the intercellular uptake of $^{99m}\text{TcO}_4$ is highly dependent on the type of cells despite no significant different of uptake was found between normal and cancer cell lines. The level of radioactivity is also an important determinant factor that influence the uptake of $^{99m}\text{TcO}_4$ into the cells. This study will be the first precedent toward understanding the cellular characteristic and pharmacokinetic of non-invasive imaging tracer for future molecular imaging and therapy.

ABSTRAK

Kajian ini bertujuan untuk mengenal pasti kadar penyerapan intersel technetium-99m pertechnetate ($^{99m}\text{TcO}_4$) oleh jenis sel yang berbeza. Kumpulan sel HeLa, sel human fetal osteoblast (hFOB), sel glial dan sel glioma dikultur dalam piring kultur dan diinkubasi bersama 200, 400, 600, 800 and 1000 μCi $^{99m}\text{TcO}_4$ selama 30 minit (37°C , kelembapan atmosfera CO_2 5%). Selepas proses inkubasi, sel dibasuh dengan phosphate buffer saline untuk membuang sisa-sisa ekstrasel $^{99m}\text{TcO}_4$. Pengukuran radioaktiviti $^{99m}\text{TcO}_4$ intersel dilakukan menggunakan kamera gamma, kemudian peratusan serapan $^{99m}\text{TcO}_4$ oleh sel-sel dikira. Hasil kajian menunjukkan kadar serapan intersel $^{99m}\text{TcO}_4$ berkadar songsang dengan radioaktiviti. Sel HeLa menunjukkan kadar serapan yang lebih tinggi berbanding sel hFOB, diikuti dengan sel glial dan sel glioma. Didapati tiada perbezaan kadar serapan antara sel kanser dan sel sihat. Konklusinya kajian ini menunjukkan bahawa kemungkinan kadar serapan intersel terhadap $^{99m}\text{TcO}_4$ sangat bergantung kepada jenis sel, namun tiada perbezaan signifikan ditunjukkan apabila sel sihat dan sel kanser dibandingkan. Paras radioaktif juga merupakan factor yang penting dalam mempengaruhi serapan $^{99m}\text{TcO}_4$ oleh sel.

Keywords: Technetium-99m-pertechnetate, In-vitro, molecular imaging

INTRODUCTION

Cancer detection through variety of medical imaging procedures such as scanning using magnetic resonance imaging (MRI), computed tomography (CT) scanner, single photon emission computed tomography (SPECT) and positron emission tomography (PET) provide different information and details on the degree of malignancy [1]. Screening cancer by employing radionuclide and appropriate radiotracer to identify diseases not only detect the location of the disease but also the physiology of the abnormality that can significantly impact the cancer patient management [2]. The details of the diseases at cellular level are vital for the accurate diagnosis and treatment prescription [3]. Radionuclides such as technetium-99m-pertechnetate ($^{99m}\text{TcO}_4$) has been used as a probes to understand the biological characteristic of the cancer cells by visualization, characterization and quantification of pathophysiological processes at the cellular and subcellular levels [4]. The interaction between cells and radiopharmaceutical, allows non-invasive detection and imaging of the cell growth and proliferation throughout the body which has long been recognised to be of significant value in the diagnosis and staging of cancer [5]. In this study, we determined the intercellular uptake of $^{99m}\text{TcO}_4$ by different types of cell lines and compare the uptake of different activity level and time of incubation. We also sought the correlation between the cell uptake and cell viability of the normal and cancerous type of cells.

MATERIALS AND METHODS

Materials.

All general chemical reagents and tissue culture reagents were purchased from Gibco, Life Technologies (USA) . The radionuclide ^{99m}Tc , was obtained from a molybdenum-99-technetium-99m (^{99}Mo - ^{99m}Tc) generator located in the Nuclear Medicine, Oncology and Radiotherapy Department, School of Medicine, Universiti Sains Malaysia. The generator ELUMATIC III was purchased from the CIS Bio International (France). The Symbia-E gamma camera (Siemens Medical Solutions, Illinois, USA) was used to measure the count of $^{99m}\text{TcO}_4$ uptake by cells.

Cell culture and culture media.

The experiments were conducted using four types of cell lines: glial cells (SVG p12), glioma (DTBRG-05MG), HeLa and human fetal osteoblast cell (hFOB). Glial, glioma and hFOB cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) while HeLa cells were grown in Roswell Park Memorial Institute (RPMI) 1640 culture media with 10% Fetal Bovine Serum (FBS), 100 units/ml penicillin and 100 μg /ml streptomycin. All cells were incubated at 37°C and 5% CO_2 humidified atmosphere. The cells were grown in 75 ml flask until confluence and were harvested for experiments using trypsin-EDTA. The trypsinized cells were plated in 6 well plates and were incubated for 24 hours before the experiments.

Preparation of $^{99m}\text{TcO}_4$.

The $^{99m}\text{TcO}_4$ were prepared from $^{99}\text{Mo}/^{99m}\text{Tc}$ generator, ELUMATIC III which produced an elution of a clear and colourless solution of sodium pertechnetate. The volume of the eluted $^{99m}\text{TcO}_4$ solution was around 5 ml with radioactivity of 200, 400, 600, 800 and 1000 μCi . The activity was measured and verified using a dose calibrator.

Determination of $^{99m}\text{TcO}_4$ uptake into cell cultures

The cells were incubated with $^{99m}\text{TcO}_4$ of different activities at 30 minutes, 1 hour and 1.5 hours of incubation time. After incubation, the culture media were removed and the radioactivity in the culture media were

counted using a dose calibrator. The cells were then washed three times with phosphate buffer saline (PBS) to remove the remaining $^{99m}\text{TcO}_4$ on the cell monolayers. Cells were detached from the culture plate by adding 0.5 ml of trypsin-EDTA and then cell were re-suspended in fresh media. The cell suspension were then centrifuge at 15,000 RPM for 5 minutes. The $^{99m}\text{TcO}_4$ uptake by the cells was measured using gamma camera and the result was expressed as the counts per minute (CPM). After the uptake measurement, the cell viability assay using trypan blue exclusion method were performed to determine the percentage of cell viability. The experiment was performed twice to confirm the reproducibility of the result.

RESULTS AND DISCUSSIONS

The data illustrated in figure 1 shows that, the maximum uptake by hFOB cell occurs at the 1.5 hours of incubation. There are no differences of uptake between 0.5 hours to the 1 hours. Highest percentage uptake were observed at the lowest activity of $^{99m}\text{TcO}_4$ and percentage uptake were decreasing with increasing activity. The data in the figure 2 shows the similar trend in $^{99m}\text{TcO}_4$ cellular uptake by glial and glioma with the hFOB cell line. The intercellular uptake for this both types of cells are relatively maximum at the lowest activity and decreased with increasing activity of $^{99m}\text{TcO}_4$. The glial recorded the higher percentage uptake at $9.44 \pm 0.09\%$ than the glioma cell line with $7.44 \pm 2.12\%$ percentage uptake at 200 μCi activity. However, the percentage uptake at higher activity show no significant differences. The figure 3 summarise the percentage uptake of all four cells lines at different activity of $^{99m}\text{TcO}_4$. The HeLa cell line shows the highest percentage uptake, $11.21 \pm 0.69\%$, while the lowest uptake was observed at $7.44 \pm 2.12\%$ for glioma. This is followed by hFOB and glial with percentage uptake of $10.02 \pm 1.41\%$ and $9.42 \pm 0.09\%$, respectively. Correlation between intercellular percentage uptake and cell viability are presented in figure 4. High cell viability increase the intercellular uptake of the $^{99m}\text{TcO}_4$ and when the viability is low, its lead to the low intercellular uptake of $^{99m}\text{TcO}_4$.

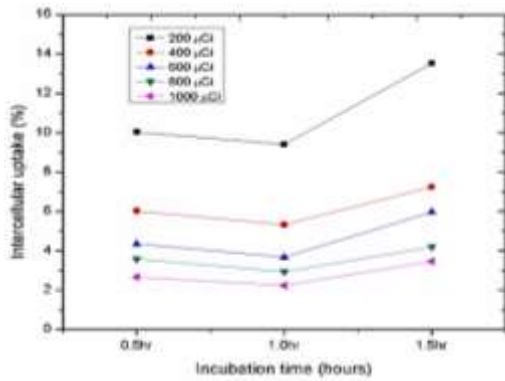


Figure 1: Intercellular percentage uptake at different $^{99m}\text{TcO}_4$ incubation time for hFOB cell line.

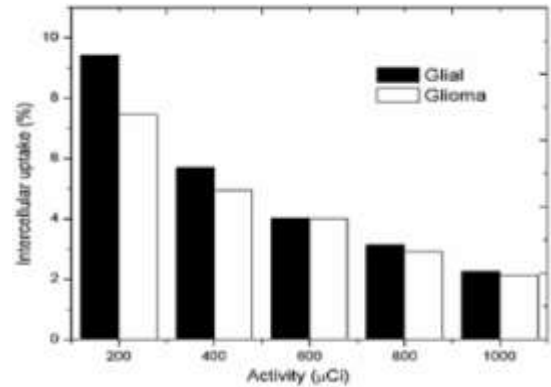


Figure 2: Intercellular percentage uptake of $^{99m}\text{TcO}_4$ by Glial and Glioma.

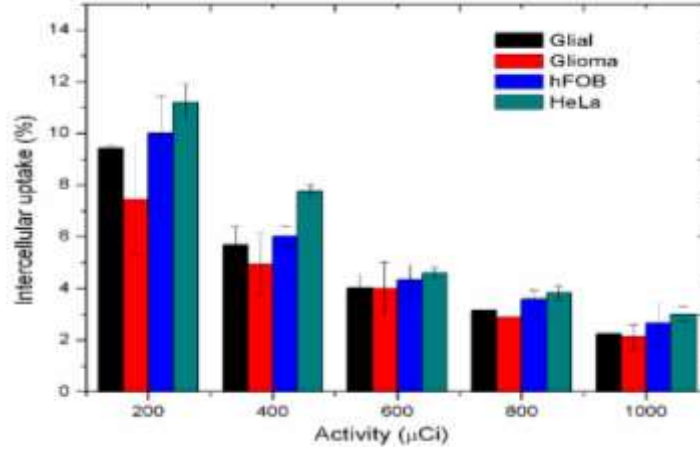


Figure 3: Intercellular uptake of $^{99m}\text{TcO}_4$ for different types of cell lines. The measurement were performed after 30 minutes incubation at 37°C and humidified with 5% CO_2 .

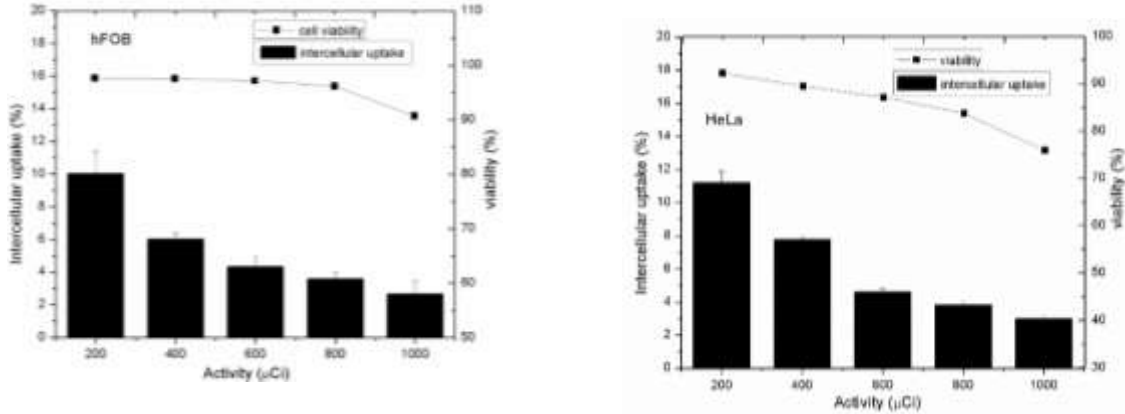


Figure 4: Intercellular hFOB and HeLa uptake in correlation with cell viability. The decreased viability of cell lines will affect the uptake percentage of both cancer and normal cell lines.

The results indicate that the intercellular uptake were maximum at lowest activity for all type of cells and longer incubation time have no significant effects. Optimal cell incubation time were found to be around 30 minutes and longer incubation time may affect the radiation counts as a results to the short half-life of $^{99m}\text{TcO}_4$. The intercellular uptake of $^{99m}\text{TcO}_4$ by different type of cells linked to the characteristic of the cells such as metabolic activity, cellular function and doubling time. Cancer cell such as HeLa is rapidly dividing type of cells were observed to have more uptake of $^{99m}\text{TcO}_4$ compare to slow dividing cell such as hFOB. However, comparison between normal and cancerous brain cells shows no significant difference in the uptake probably linked to other factor such as drug resistant characteristic and physiological parameters such as plasma membrane potential and intracellular pH [6, 7, 8].

CONCLUSION

The findings of this study suggest that the intercellular uptake of $^{99m}\text{TcO}_4$ is highly dependent on the type of cells despite no significant different of uptake was found between normal and cancer cell lines. The level of

radioactivity is also an important factor that influences the uptake of $^{99m}\text{TcO}_4$ into the cells. The results also shows correlation between the cellular uptake and the cell viability. Further studies need to be conducted to confirm the relationship between radiotracer uptake and the cellular characteristics of the cells.

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